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Challenges in biobutanol production: How to improve the efficiency?

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ABSTRACT

There is an increasing interest in the production of chemicals and fuels from renewable resources due to the continuing price increase of fossil resources, the insecurity of the availability of fossil resources in the future, and additionally environmental concerns and legislations. Biobutanol may be produced by the acetone–butanol–ethanol (ABE) fermentation.

This paper reviews the biobutanol production bringing up the problems and challenges to overcome. The aim of the paper is to help in finding opportunities to make the process feasible in the near future. The analysis stresses the idea of improving the efficiency of the fermentation stage by altering the up (pretreatment of the raw material) and downstream (product recovery and purification) processes. The paper also explores the biobutanol production from the biorefinery perspective. Finally the review brings up the important role of research in developing and implementing the production of biobutanol by the ABE fermentation.

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Abbreviations: AB, acetone–butanol; ABE, acetone–butanol–ethanol; CS, chitosan; FCDA, furan-2,5-dicarboxylic acid; GFT, Gesellschaft für Trenntechnik; HFM, 5-hydroxymethylfurfural; HMR, hydroxymatairesinol; IL, ionic liquid; PAI, polyamide-imide polyetherimide; PAN, polyacrylonitrile; PEBA, polyether block amide; PEI, polyetherimide; PDMS, polydimethylsiloxane; PEP–PTS, phosphoenolpyruvate dependent phosphotransferase system; PPS, polyphenylene sulfide; PTMSP, poly(1-trimethylsilyl-1-propyne); PVA, poly(vinyl alcohol); PVDF, polyvinyledenefluoride; SA, sodium alginate; $scCO_2$, supercritical carbon dioxide.

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1. Introduction

During the last decade there has been an increasing interest for the production of chemicals and fuels from renewable resources [1]. Reasons for this trend include growing concerns about global warming and climatic change, volatility of oil supply, increasing crude oil price, and existing legislations restricting the use of non-renewable energy sources. Further, the generation of biofuels may improve the local employment opportunities and contributes to the reduction of CO_2 emissions.

Butanol is used as a solvent, in cosmetics, hydraulic fluids, detergent formulations, drugs, antibiotics, hormones and vitamins, as a chemical intermediate in the production of butyl acrylate and methacrylate, and additionally as an extractant in the manufacture of pharmaceuticals [2]. However, it may also be used as a fuel. Butanol, as compared to ethanol, is less volatile and explosive, has higher flash point, and lower vapour pressure, which makes it safer to handle. It contains more energy, it is less hygroscopic (thus does not pick up water) and can easily mix with gasoline in any proportion [3]. In addition, the air to fuel ratio and the energy content of butanol are close to gasoline. Butanol can be used directly or blended with gasoline or diesel without any vehicle retrofit and supplied through the existing gasoline pipes [4]. When considering butanol as a fuel, it is also important to evaluate its hazardous characteristics. Butanol contributes to the formation of photochemical smog when it reacts with other volatile organic compounds in the atmosphere [2]. Butanol is flammable and causes irritation of eyes, nose and throat in humans [5].

Butanol is mainly produced via chemical synthesis. The most common chemical route is the Oxo process, which involves the reaction of propylene with carbon monoxide and hydrogen in the presence of an appropriate catalyst. The resulting mixture of *n*- and isobutyraldehyde is hydrogenated to the corresponding *n*- and isobutyl alcohols [6] and further distilled to recover butanol in the required quality.

$$CH_3CH{-}CH_2 + CO + H_2 \rightarrow CH_3CH_2CH_2CHO + (CH_3)_2CHCHO \quad (1)$$

$$CH_3CH_2CH_2CHO + H_2 \rightarrow CH_3CH_2CH_2CH_2OH \quad (2)$$

Other fossil oil derived raw materials used in the production of butanol are ethylene, propylene and triethyl aluminium or carbon monoxide and hydrogen [7].

Butanol can also be obtained from renewable resources (biomass) by the acetone butanol ethanol (ABE) fermentation (Fig. 1). This process is called biobutanol or the ABE production. Butyric and acetic acids are first produced by *C. acetobutylicum* (acidogenesis), and in the subsequent phase (solventogenesis) butanol, acetone and ethanol are formed. The biobutanol production consists of several stages (Fig. 2): prior to the ABE fermentation the biomass consisting of starch rich, sugar rich or lignocellulosics materials is pretreated in the upstream processing and used as a substrate. The pretreatment method differs depending on the type of biomass used. After the fermentation the desired product is recovered and purified in the downstream processing. Biobutanol production was already developed in the 1910s and ceased to be in use after already twenty years. The use of this process declined due to economic reasons as the petrochemical industry evolved.

The economics of the biobutanol production are largely dependent on the cost of the fermentation substrate. For biobutanol to have a meaningful impact as an alternative fuel, the biomass feedstock must be widely available at low cost [8]. Effective bioconversion of the feedstock as well as having the lowest possible environmental impact in its use and production are also key issues. Other negative aspects that made producing butanol from petrochemicals more competitive than via bioprocesses were the low yield encountered, sluggish fermentations, uneconomical product recovery caused by low butanol titer and purity in the fermentation broth and problems with the degeneration of production organism, and phage infections.

Research plays a vital role in making the production of biofuels feasible. Improvements and developments in process and bioprocess technologies, in applied microbiology and genetic engineering could result in an improvement of the economic competitiveness of the fermentation route for biobutanol production. Areas of ABE fermentation under research and development are alternative substrates for fermentation [9,10], removal of inhibitors present in biomass hydrolyzates [11,12], product recovery [13–15], improvement of analytical methods for process monitoring [16,17] and metabolic engineering of *Clostridium* species and other potential producers [18,19].

The industry also shows interest in biobutanol production. In 2007 Green Biologist Ltd publicized a patented hydrolysis technology to be integrated into the biofuel fermentation process to reduce the feedstock and manufacturing cost [20]. The company works in advances in continuous fermentation and integrated recovery to drive down costs further [21]. DuPont and BP are focused on developing biocatalysts to produce 1-butanol as well as 2-butanol and iso-butanol. The first commercial scale biobutanol facility is expected to begin operation in 2013 [22]. Cobalt Technologies [23] has recently announced the opening of its first biobutanol production facility.

This paper presents the production of butanol by the ABE fermentation and analyses each step of the process while bringing up the problems and challenges to overcome. The analysis stresses the idea of improving the efficiency of the fermentation stage by altering the up (pretreatment of the raw material) and downstream (product recovery and purification) processes. The paper also explores the butanol production from the biorefinery perspective. The purpose of the present paper is to review the ABE fermentation in order to find opportunities to reduce the production costs so that the process will be economically feasible in the near future.

2. History of biobutanol production

The formation of butanol in the microbial fermentation was reported first by Louis Pasteur in 1861 [24]. In the following years Albert Fitz worked actively in the field of fermentation and obtained butanol from glycerol using a mixture of two bacteria [25]. Among others, researchers continuing with this work were Beijerinck, Bredemann Shardinger and Pringsheim [24].

In the beginning of the 20th century, the research was focused on producing acetone, amyl alcohol or butanol by fermentation to use them for the manufacture of synthetic rubber [24,26]. The company Strange & Graham Ldt. employed Professor Perkin and his assistant chemist Weizmann from Manchester University as well as Professor Fernbach and his assistant Schoen from the Pasteur Institute to work in this research field. In 1911 Fernbach isolated and patented a culture that enabled the fermentation of butanol from potato starch. This process was industrially implemented in Rainham during 1913–1914 and further transferred to another plant of Strange and Graham Ldt. at King's Lynn [26]. In the summer of 1912, Chaim Weizmann left the research group and started his own studies. He succeeded in isolating a bacterium strain (later named *Clostridium acetobutylicum*), which was capable of using starch as a substrate in the butanol production process [26,27]. This process exhibited higher product yields of butanol and acetone and replaced Fernbach's process. The need of cordite (smokeless gunpowder), the production of which requires acetone, during the First World War [26] made acetone the desired product of the fermentation process. At that time, butanol was considered only a byproduct

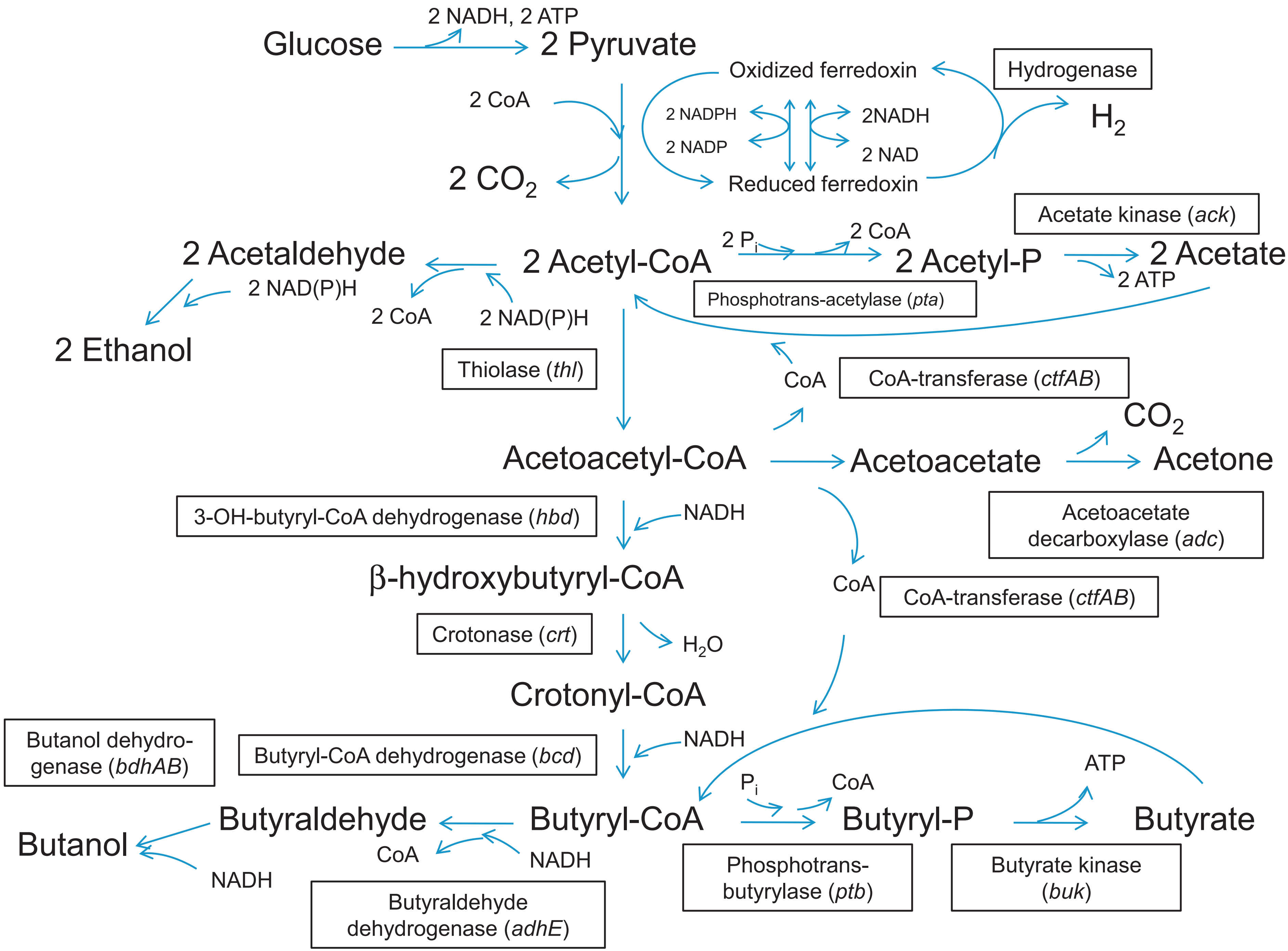


Fig. 1. Acidogenic and solventogenic metabolic pathways in clostridia. The enzymes involved are shown in boxes with the corresponding gene in parenthesis.

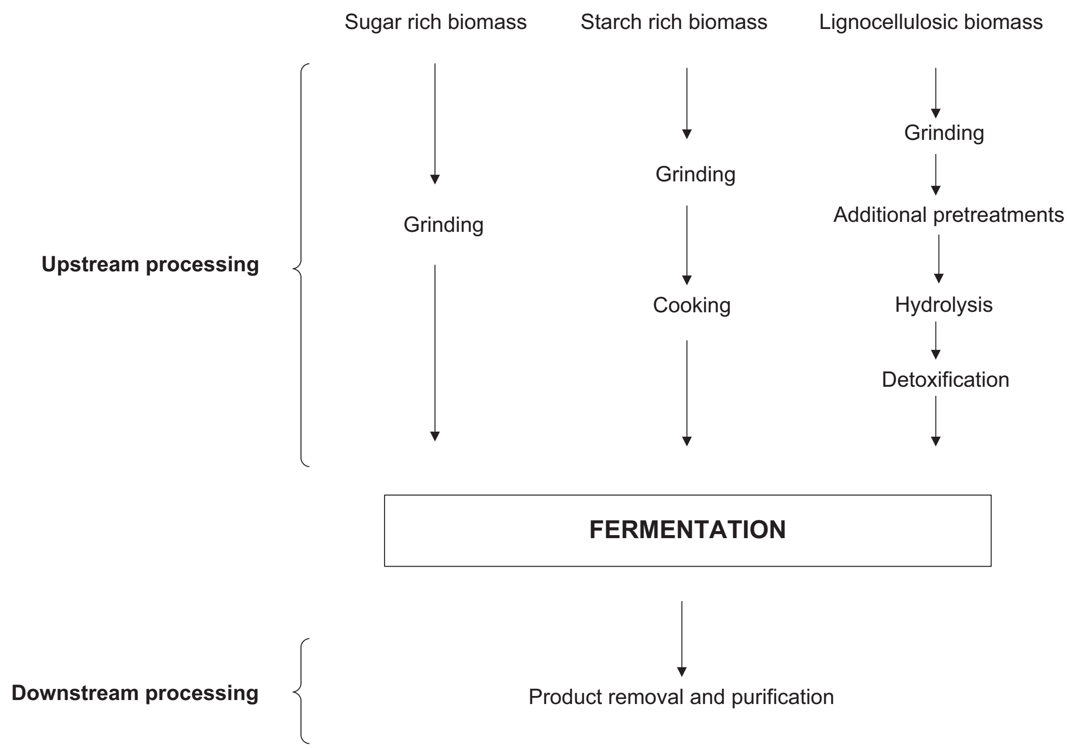


Fig. 2. Schematic diagram of the production of butanol from biomass.

and was stored in containers. The expanding automobile industry needed a solvent for the quick drying lacquers for the finishing of cars. Butanol and butyl acetate showed good properties as solvents for this purpose and in 1920 butanol became the main product of the fermentation process [24,26]. The ABE process was successful prior to the actual development of large scale, aseptic, submerged industrial fermentation technology. This may be explained by the nature of the fermentation (anaerobic) and the products (toxic acids and solvents).

Several countries produced biobutanol at industrial scale during the period 1920–1980 (Table 1) [7,24,26–34]. For the processes locally isolated clostridium strains were used. Since 1911 new discoveries are continuously patented until present [4]. Research and development was especially intensive in the USSR where the biobutanol plant in Dokshukino focused on process development. New strains were isolated and novel substrates and more effective preparation methods were developed. The fermentation step was improved by a better equipment design and by using parallel batteries of reactors in series. Continuous processes and exploitation of by products were carried out as well [7]. However, many plants were forced to be closed during the 1960s due to the increased price of substrates, low solvent yields and a more competitive process based on fossil fuels [34]. The industrial biobutanol production ceased in the early 1990s [27]. Research and development of the ABE process continued and during the 1980s and 1990s studies at pilot plants were carried out in Soustons, France and in Lower, Austria. In France, the research focused on evaluating the commercial potential of solvent production for gasoline substitute fuels by using hydrolysates of cereal straw, corn stover and sugar beet in batch fermentation. Potatoes were used as raw material for batch, fed batch and continuous fermentation modes in Austria [29].

China developed its ABE process industry from *C. acetobutylicum* strains using mainly starch based feedstocks [32]. The first plants were built in the 1960s reaching an annual production of 170,000 tons. However, the facilities were closed at the end of the 20th century due to the rapid development of petrochemical synthesis processes. In the past years China has restarted the ABE production. At least 11 production plants are in operation and others are under construction or at the start up stage [33].

3. Production of butanol by fermentation

Several species of *Clostridium* bacteria are capable of metabolizing different sugars, amino and organic acids, polyalcohols and other organic compounds to butanol and other solvents. Butanol being of relatively high value is usually the most desired product. Depending on the strains and substrates used in the fermentation, the optimal fermentation conditions (pH, temperature, nutrients), fermentation products and the ratio of their formation vary.

Research on the butanol production by fermentation is mainly focused on the field of understanding the physiological principles of solvent production and metabolic engineering. Relatively few species of clostridia (including the mutants from the most well known species) have been used in research. This may be explained by the fact that while searching for high conversion of glucose to butanol, the best strains already seem to reach the limits of butanol tolerance. Conventional clostridial fermentation processes with the best natural strains produce butanol titers ca. 13 $g\,l^{-1}$, which is often stated as the upper limit tolerated by the natural strains. Butanol tolerance should naturally be one of the topics in research through strain development and/or innovative process approaches in fermentation. However, there is a lack of data and understanding of the butanol tolerance in microbes at genetic and protein level.

The number of metabolic engineering studies related to the production of butanol by fermentation has increased since the 1990s. The genome data from the two clostridial strains most widely studied for the butanol production, *C. acetobutylicum* ATCC 824 and *C. beijerinckii* NCIMB 8052, is used to construct strains with genes knocked out and/or overexpressed. The most common targets are to increase the production of butanol compared to the other solvents, to increase the butanol titer and purity of the product in

Table 1
Production of biobutanol in industrial scale during the period 1920–1980s.

Production years	Location	Substrate	Notes	Reference
1913–1914	Rainham, England	Potato starch	"Fernbach's process"	[24]
			Fermentation by a mixture of bacteria	
1916–1918	Toronto, Canada	Starch	"Weizmann's process"	[26]
			Total production 3000 t of acetone and 6000 t of butanol	
1917–1918	Terre Haute, USA	Corn starch, molasses	Two distilleries in operation	[24,26]
1920–1935			Butanol as the main product from 1920	
			9 months of shutdown in 1920 because of business decline	
			Bacteriophage infection in 1923	
1923–1935	Peoria, USA	Corn starch, molasses	Built up because of the bacteriophage infection in Terre Haute and the good availability of substrates (corn)	[24,26,28]
			96 units, total volume about 18,168 m^3	
			Production of methanol from the fermentation gases by catalysts	
1930s	Philadelphia and Puerto Rico in USA, Egypt, India and Australia	Molasses	Use of phage-immunized *C. saccharobutylicum* strains capable of metabolizing sucrose	[29]
			C. madisonii used in 1940s in Puerto Rico	
1940s–1960s	Japan	Molasses	*C. saccharoperbutylacetonicum* used	[28]
1929–1935[a]	Dokshukino, Grosnyi and Talitsk, USSR	Wheat and rye starch, wheat flour, molasses, pentosane hydrolysates from hemicelluloses	Total capacity of Dokshukino plant: 6500 m^3	[7]
			Parallel batteries of reactors in series to increase site production	
			1958–1962: development of continuous substrate preparation and distillation with batch fermentation	
			1959–1961: development of a pentose hydrolysis and fermentation	
1935–early 1950s	Bromborough, England	Molasses	Strains of *C. saccharobutylicum* used	[30]
1936–1982	Germiston, South Africa	Maize mash, molasses[b]	Strains P265 and P270 used in the 1970s	[27,28,31]
			Several phage infections detected during the production years	
1960[c]	Shanghai, Beijing, and Wuxi, China	Starch	Yearly production of 1000 t in each plant	[32]
1965–1970[c]	Zhejiang, Tianjin, Yunnan, and Shanxi, China	Starch substrates (corn, corn starch, cassava)	Production capacities 3000–10 000 t/year	[32,33]
1980s[c]	Hebei and Shandong, China	Starch substrates (corn, corn starch, cassava, sweet potato)	Total annual solvent production of 170 000 t reached	[32]
1947–1957	Formosa, Taiwan	Molasses	Strain of *C. toanum Baba* used	[34]

[a] Some of the plants worked until the end of the 1980s.
[b] The substrate was used after 1944.
[c] Building up year of the plant.

the final fermentation broth, to speed up the fermentation process by omitting the separate acidogenic phase, and to simplify and intensify the fermentation by avoiding the coupling between sporulation and solvent production. The most common targets are (1) to increase the production of butanol compared to the other solvents, (2) to increase the butanol titer and purity of the product in the final fermentation broth, (3) to speed up the fermentation process by omitting the separate acidogenic phase, (4) and to simplify and intensify the fermentation by avoiding the coupling between sporulation and solvent production. As the molecular biology tools for clostridia have been lately developed, truly cellulolytic strains are constructed by expressing heterologous genes for various cellulolytic enzymes in butanol producers. A new approach for the strain development is the metabolic engineering of both prokaryotic and eukaryotic species, which do not produce naturally butanol. Butanol titers with these strains have been modest so far (approximately 1 $g\,l^{-1}$ or less).

Fermentation with free cells produces a broth with undissolved solids containing cells and possible nutrient sources, and several components in solution. Both butanol concentration and purity in the broth are often low. Butanol is recovered and purified in a downstream processing stage. After typical batch fermentation the microbial cells may be separated by centrifugation or filtration. The fermentation broth may also be distilled with the solids in it similarly to the case of ethanol production. If a part of the cellmass is recycled back into the fermentor, the cells in the broth should be first concentrated by membrane separation or by centrifugation. With immobilized cells a separate solid/liquid separation stage may be omitted. There are several options for the recovery and purification of butanol and this is discussed in Section 3.6.

Improving the economics of the butanol production by fermentation is crucial. However, there are only a few studies available on this topic. Gapes [35] presented calculations on the economics of the combined fermentative acetone and butanol (AB) production. Assuming 0.5€ kg^{-1} as a price for both products, it was estimated that a break even point is achieved in a grass roots plant at a fermentation substrate (carbon source) price of 0.05–0.09€ kg^{-1}. The average price for butanol during the period 1995–2000 was 0.61€ kg^{-1} with 29% annual variation [35]. However, the price in the US has increased steadily during this century being approximately 1.5$ kg^{-1} in the beginning of 2006 [36]. Lignocellulosic biomass is considered a cheap substrate and the production of butanol based on lignocellulosic materials has been a key topic within research on biofuels fermentation since the late 20th century. In spite of the importance of improving the volumetric productivity (as grams of product produced/liter and hour) not much attention has been paid to it. Typical volumetric productivities in the conventional ABE fermentation (ratio of the products 3:6:1) are low ($<0.5\,g\,l^{-1}\,h^{-1}$) even compared to those in other fermentations for bulk chemicals ($2–4\,g\,l^{-1}\,h^{-1}$). The maximum stoichiometric yield of butanol from glucose is 0.411 $g\,g^{-1}$ [37].

3.1. Physiology

An important feature of Clostridia in relation to the process economics is their ability to metabolize both hexose and pentose sugars through glycolysis and the nonoxidative pentose phosphate pathway. The butanol synthetic pathway in *Clostridium* species starting from pyruvate is shown in Fig. 1. The oxidative pentose phosphate pathway is not annotated in *C. acetobutylicum* [37]. Clostridia have phosphotransferase systems (phosphoenolpyruvate dependent phosphotransferase system, PEP–PTS) for the uptake of most of the fermentable sugars. In addition, some species have nonPTS uptake systems the role of which may be pronounced in the solventogenic phase [38]. During the acidogenic phase clostridia grow and produce mainly acetic and butyric acids, CO_2 and H_2 and lower the pH of the medium.

Hydrogen production may be regarded as a waste of electrons/energy that could otherwise be used in the solventogenic phase, which as such is in the reduction oxidation balance. Gassing with CO is known to reduce hydrogen formation by inhibiting hydrogenase and to increase the electron flux from the reduced ferredoxin (FdH_2) to NAD^+. Long et al. [39] reported that *C. acetobutylicum* P262 consumed 24–40% of the sugar (60 g glucose l^{-1}) during the acidogenic phase producing 4–6 $g\,l^{-1}$ acids. The phase shift was accompanied with sporulation (70–80% of the cells) and cease of growth. At pH 5.5, the solvent production started while the acid concentration remained almost constant. Later reports show some acid consumption during the solventogenic phase, but a part of the acids remains in the final fermentation product lowering the butanol purity in the broth. Sporulation and solvent production seem to be interlinked as Long et al. [39] have shown loss of solvent production with the isolated sporulation mutants.

The concentration of the undissociated butyric acid seems to trigger the phase change from the acidogenic to the solventogenic phase. Richter et al. [40] could induce a solventogenic phase even at pH 6 or higher if the concentration of butyric acid was high enough. Ballongue et al. [41,42] claimed that the acids act as inducers for the key enzymes necessary for the butanol biosynthesis. More exact data was presented by Terracciano and Kashket [43] and Husemann and Papoutsakis [44] giving 13–18 mM and 6–13 mM, respectively as the triggering undissociated butyric acid concentrations. Starting from the late 1990s molecular biology tools for clostridia were successfully developed enabling the use of strains with specific inactivated and/or overexpressed genes for more comprehensive metabolic studies. Harris et al. [45] showed that certain intracellular metabolic precursors such as butyryl-CoA and/or butyrylphosphate might be the key components in the phase shift. There exist only a few studies on the transcriptional regulation of the genes encoding enzymes of the solventogenic pathway some of which form operons (*sol* operon) in several clostridia. The most common transcriptional factor in clostridia is Spo0A which is involved in the initiation of both the sporulation and solvent formation in e.g. *C. acetobutylicum*. Kosaka et al. [46] found an extracellular peptide acting as a transcription factor for the operon and this peptide was produced by *C. saccharoperbutylacetonicum* N1-4 itself. While studying the phase shift Nair et al. [47] cloned a s*olR* gene encoding a repressor protein in *C. acetobutylicum* 824. Overexpression of the s*olR* prevented most of the solvent production with a small effect on the acid formation. The strain with the inactivated *solR* gene produced 27 $g\,l^{-1}$ solvents (17.8 $g\,l^{-1}$ butanol) with no clear acidogenic phase. On the contrary to aerobic spore formers glucose and/or ammonia limitation is not triggering spore formation in clostridia. Accordingly nutrient limitation does not trigger the shift to the solventogenic phase either.

The entire genomes of *C. acetobutylicum* ATCC 824 [48] and *C. beijerinckii* NCIMB 8052 [49,50] have been sequenced and at least that of *C. saccharoperbutylacetonicum* N1-4 [46] is currently being worked out. Genomics has already enriched physiological studies of solvent production in these strains and their mutants – induced with either directed or random mutagenesis. As an example Shi and Blaschek [50] compared the expression of ca. 500 genes, solvent formation and sporulation events in *C. beijerinckii* NCIMB 8052, its solvent hyperproducing mutant BA101 and *C. acetobutylicum* ATCC 824. The sporulation events took place more rapidly in *C. beijerinckii* NCIMB 8052 than in *C. acetobutylicum* ATCC 824. The parent strain *C. beijerinckii* NCIMB 8052 as compared to the mutant BA 101 showed lower levels of expression of several butanol pathway genes and a more efficient sporulation and phosphotransferase system for sugar transport.

3.2. Metabolic engineering

Metabolic engineering studies may be divided into those on natural butanol forming clostridia and the ones aiming at setting up butanol synthesis pathways in organisms not naturally producing butanol. The general targets of the metabolic studies of clostridia are: (1) tolerance of a strain to oxygen, (2) growth to much higher cell densities, (3) prolonged cell viability, (4) direct utilization of cellulosics, (5) an asporogenous producer, (6) a stable (nondegenerating) strain, (7) high solvent tolerance, and (8) high butanol selectivity [51]. Lee et al. [4] summarized the development of genetic tools for clostridia as well as titers of different products produced by various up and/or downregulated genes in *C. acetobutylicum* strains. The highest titers of butanol were achieved with the *solR* knock out mutant (17.8 g l^{-1}), with a strain overexpressing the heat shock protein groESL (17 g l^{-1} in [52]) and with the strain having the gene for the butyrate kinase knocked out (16.7 g l^{-1} in [45]).

Sillers et al. [53] presented an interesting approach to avoid the formation of acetone, to generate nonsporulating yet butanol producing strains, to increase the butanol productivity, and to enable the application of fed batch and continuous fermentation modes. The megaplasmid pSOL1 carries the *sol* operon genes for the solvent producing enzymes alcohol/aldehyde dehydrogenase (*aad*), CoA-transferase (*ctfA* and *ctfB*) and acetoacetate decarboxylase (*adc*). In their approach, the researchers used the aad gene to complement a *C. acetobutylicum* strain, which has previously lost the megaplasmid [53]. This complementation restored the production of butanol, but in spite of several other modifications at the genetic level, the butanol titer was moderate (ca. 9 g l^{-1}) with rather high concentrations of acids. However, no acetone was produced. Jiang et al. [54] improved the selectivity of the butanol production compared to other solvents in *C. acetobutylicum* by inactivating the gene for the acetoacetate decarboxylase. This resulted in a low acetone titer (approximately 0.3 g l^{-1}) but increased the production of acetic acid. Wild type butanol concentrations (ca. 14 g l^{-1}) were achieved with the ratio of butanol to total solvents of 4:5 by controlling the pH of the medium.

It is common for clostridia to produce hydrolytic enzymes towards lignocellulosic materials. Cellulolytic enzymes are organized in a multienzyme complex with several catalytic components around a scaffold protein. This complex is called cellulosome. *C. acetobutylicum* ATCC 824 can produce a cellulosome, but is not able to degrade crystalline cellulose or grow on lignocellulosic materials. *C. beijerinckii* NCIMB 8052 possesses no cellulosome genes and it seems incapable of hydrolyzing xylan [49]. *C. acetobutylicum* ATCC 824 instead grows slowly on xylan [55]. Cellulase genes from both clostridial [56] and fungal [55] species have been expressed in clostridia resulting in low activity levels and no utilization of crystalline cellulose. The possible reasons for these results are a missing, critical element or its expression, or too low activity levels of the enzymes. It is, however known that several butyric acid producing clostridia are able to utilize cellulosic materials, and research is active within this topic.

In order to avoid problems with clostridia several new approaches based on metabolic engineering of other microbes are under investigation with a growing interest. Some of these approaches bring along new products such as longer chain and branched chain alcohols. For example, 2-butanol is regarded as a better biofuel than 1-butanol (i.e. *n*-butanol) because of the lower boiling point of the former (98 °C vs. 118 °C) and the higher tolerance of the host bacteria towards 2-butanol than 1-butanol (40 g l^{-1} vs. 20 g l^{-1}) [57].

Atsumi et al. [18] expressed in *E.coli* 6 clostridial butanol pathway genes coding for thiolase (*thl*), 3-OH-butyryl-CoA dehydrogenase (*hbd*), crotonase (*crt*), butyryl-CoA dehydrogenase (*bcd*), electron transferring flavoprotein (*etf*) and alcohol/aldehyde dehydrogenase (*adhE2*) in addition to overexpression of homologous acetyl-CoA acetyltransferase (*ato*). By deleting several *E. coli* genes coding for enzymes responsible for side products, a butanol titer of ca. 0.35 g l^{-1} was achieved. On a rich fermentation medium with glycerol as the carbon source (20 g l^{-1}) a titer of approximately 0.55 g l^{-1} was reached. A similar approach was used by Inui [58] reaching approximately for 1.2 g l^{-1} of butanol from 40 g l^{-1} of glucose. A similar type of a strain construct was made by Steen et al. [59] using *Saccharomyces cerevisiae* as the host. Different sets of genes were evaluated for butanol formation and the highest butanol titer achieved was 0.0025 g l^{-1} from 20 g l^{-1} galactose. Some lactic acid bacteria (e.g. *Lactobacillus delbruckii* and *Lactococcus lactis*) are known to grow in the presence of 3 wt% butanol. Liu et al. [60] thus expressed the *C. beijerinckii* gene for thiolase in *L. lactis* and *L. buchnerii.* Both constructs formed butanol and the highest butanol concentration of 0.066 g l^{-1} was achieved with *L. buchnerii.* Some *Pseudomonas putida* and *Bacillus subtilis* strains can tolerate up to 6% (vol/vol) and 4.5% (w/v) of butanol, respectively. That property was the rationale for the engineering of these bacteria for butanol production by expressing the clostridial butanol pathway genes by Nielsen et al. [61]. This study resulted in 0.12 g l^{-1} and 0.024 g l^{-1} butanol, respectively.

Finally, Atsumi et al. [62] described how *E. coli* may be engineered to produce several potential biofuel alcohols including butanol via biosynthetic pathways for certain aminoacids (threonine, valine, leucine, isoleucine, phenylalanine). Various 2-keto-acids are the key intermediates while unspecific 2-keto-acid decarboxylases and alcohol dehydrogenases are responsible for the cleavage of CO_2 and reduction of the corresponding aldehydes. In the case of 1-butanol, approximately 0.67 g l^{-1} was produced from 36 g l^{-1} glucose and 8 g l^{-1} threonine (as a cosubstrate). The isobutanol route was exceptionally efficient producing 22 g l^{-1} without any cosubstrate.

3.3. Butanol tolerance

Butanol tolerance of a bacterium used in the fermentation is an important aspect affecting the economics of the production. Low concentration of butanol in the fermentation broth causes high product recovery costs. Ezeji et al. [63] have recently reviewed the studies on butanol tolerance.

Butanol toxicity is mainly caused by its hydrophobic nature that increases the fluidity of the cell membrane. As a result, the function of the cell membrane as a controlling barrier between cell interior and exterior is affected. Additionally, the transmembrane pH gradient is destroyed resulting in energy shortage. Microbes in general react into solvent stress by affecting the fluidity of the cell membrane, by producing heat shock proteins, and by activating efflux pumps for the solvents. The fluidity of the cell membrane may be affected by increasing the share of saturated fatty acids in the membrane and by cis to trans isomerization of the unsaturated fatty acids in the membranes thus decreasing the partition of a solvent into the membrane.

Transcriptomic [64] and proteomic [65] studies were recently performed with both wild type clostridia and a mutant with increased tolerance to butanol (13 vs. 19 g butanol l^{-1}). The results are consistent with each other regarding to the synthesis of chaperones and butanol pathway enzymes (upregulation/higher protein levels) during butanol stress. This is in accordance with an increased butanol tolerance in a strain overexpressing the chaperone GroESL [42]. As Alsaker et al. [64] and Mao et al. [65] stated, this kind of studies with gram positive bacteria are scarce and it is most probable that the enhancement of butanol stress in microbes will be induced by several factors. Borden and Papoutsakis [66] listed 16 gene candidates for improving butanol tolerance by genetic

engineering. Directed and random mutagenesis, continuous cultivation, adaptation and serial enrichment will be useful tools in the future. Butanol tolerance and butanol productivity are naturally not inevitably interlinked in mutants. This is shown by the fact that some mutants with enhanced butanol tolerance produce increased amounts of butanol while some of these mutants show no change in this respect, and some even produce less butanol than the wild type. For instance, adaptation by alterations in membrane fluidity may affect induction of solventogenesis and the rates of nutrient consumption.

Knoshaug and Zhang [67] measured the butanol tolerance as relative growth rate (100% corresponding growth rate with no butanol present) of 24 microbial species. Out of the 16 yeast species tested only 4 were able to grow at the butanol concentration of 2 wt% and the growth rate was <20%. Some of the *S. cerevisiae* strains tested, however, showed a tolerance to even 14 wt% of ethanol. It is well known that the toxicity of alcohol increases with the chain length. The lower the temperature the higher is the tolerance. Lactic acid bacteria tested had higher tolerance to butanol and the *Lactobacillus brevis* strain had the highest tolerance showing a 30% relative growth rate at a butanol concentration of 3 wt%. The most tolerant *E. coli* strain tested showed 70% relative growth rate at a butanol concentration of 1 wt%, but no growth in 2 wt% of butanol.

Pseudomonas putida strains are known to tolerate exceptionally high concentrations of solvents. Ruhl et al. [68] adapted a *P. putida* strain up to 6% (vol/vol) of butanol. At this concentration the relative growth rate was approximately 10%. The adapted strain had an increased maintenance energy demand and a higher specific rate of glucose uptake than the original strain. This may be explained by an increased activity of an efflux pump induced by butanol and/or by changes in the fatty acid composition in the cytoplasmic membrane. Kieboom et al. [69] cloned the genes for a solvent efflux pump from *P. putida* S12 strain. The genes were transferred into a solvent sensitive strain resulting in an acquired solvent resistance. Such an approach may open up new possibilities to engineer the butanol tolerance especially into new engineered potential butanol producers.

3.4. Fermentation practices

The conventional clostridial process is considered as a typical batch process with only some alternative process modes. Metabolic engineering of the solvent producing clostridia to avoid an acidogenic phase has offered new approaches. This is the case also with all the engineered nonnatural butanol producers. With a typical average volumetric productivity by clostridia ($0.5\,g\,l^{-1}\,h^{-1}$) in a batch fermentation, the fermentation capacity of $25{,}000\,m^3$ would be needed for an annual production of 80,000 tons of butanol! As an anaerobic fermentation the size of a fermentor unit is not limited by oxygen or heat transfer rates. Volumetric productivity may be improved by increasing the cell density and/or by improving the specific productivity of the cells. The average volumetric productivity in a continuous fermentation may in principle be higher than in a batch process because of less downtime, but in the case of butanol with clostridia, the limited cell growth during the production would require recycling of the cells. An additional problem especially with *C. acetobutylicum* is the possible degeneration of the solvent production capability. This is a result of the loss of the megaplasmid (pSOL1) containing solventogenic genes. In *C. beijerinckii* these genes are in the chromosome and thus degeneration is less of a problem. Chen and Blaschek [70] noticed that acetate addition into the fermentation medium prevented degeneration and increased butanol production by a hyper producing *C. beijerinckii* strain BA101.

Tashiro et al. [71] succeeded in producing approximately $15\,g\,l^{-1}$ of butanol with an average volumetric productivity of $0.6\,g\,l^{-1}\,h^{-1}$ in a fed batch process (downtime not taken into account). This was achieved by feeding the reactor with a mixture of glucose and butyric acid at an optimized ratio when approximately 50% of the batch glucose was consumed. The feeding rate was adjusted to keep the broth pH constant. The feeding of butyric acid practically prevented the formation of ethanol. Later Tashiro et al. [72] used nongrowing cells for the production of approximately $9\,g\,l^{-1}$ of butanol with high yield using a mixture of glucose and butyric acid as the substrate. Lee et al. [73] also used a butyrate/glucose mixture ($2/60\,g\,l^{-1}$) as a substrate and compared continuous fermentation with free and immobilized cells. Without butyrate the production of butanol was very low. Volumetric productivity and butanol yield from glucose with immobilized cells were $0.4\,g\,l^{-1}\,h^{-1}$ and $0.44\,g\,g^{-1}$, respectively, with a dilution rate of $0.04\,h^{-1}$. These results were superior to those with free cells. It was also reported that butyrate prevented the degeneration of solventogenesis. The process was run in a steady state for over 150 days.

Mutschlechner et al. [74] set up a two stage continuous fermentation with a *C. beijerinckii* strain with glucose as the carbon source. In the first stage (acidogenic turbidostat stage), cells grew with almost a maximum specific growth rate of $0.12\,h^{-1}$ and the broth was transferred into the second stage (solventogenic stage) at the so-called acid break point. The volume of the second stage was set to end up with a specific growth rate of $0.022\,h^{-1}$. All the glucose ($50\,g\,l^{-1}$) was fed into the first stage and some 15% of it was consumed there. The final butanol titer was $9.1\,g\,l^{-1}$ and the volumetric productivity was $0.16\,g\,l^{-1}\,h^{-1}$. Production was stable for at least 1600 h.

Pierrot et al. [75] reached a high volumetric productivity for total solvents of $6.5\,g\,l^{-1}\,h^{-1}$ in a continuous fermentation where a part of the cells was recycled by using an ultrafiltration unit. This productivity figure is the highest found in the literature. The cell concentration was maintained at $20\,g\,l^{-1}$. A solvent concentration was $16\,g\,l^{-1}$ with the butanol:acetone:ethanol ratio of 8:6:1. The process was run at a steady state for 150 h.

Qureshi and Blaschek [76] evaluated the economics of three alternative fermentation modes (batch, fed batch and the use of immobilized cells) all with in situ recovery of the solvents. Corn was used as the carbon source. The costs of butanol production were 0.14, 0.12 and 0.11$\,kg^{-1}$ butanol, respectively.

3.5. Upstream processing

Upstream processing consists of the following steps: pretreatment, hydrolysis and detoxification. Selected feedstock and process conditions in the pretreatment stages determine the type and number of required upstream steps. The selection of feedstock depends on its availability, price, and the effectiveness of its pretreatment, hydrolysis and fermentation. Available feedstock materials for the bioproduction of ethanol and butanol (or ABE) are diverse. These include different kinds of by products, wastes and residues of agriculture and industry [77]. Corn, molasses and whey permeate from the cheese industry are the traditional raw materials for the process. Lignocellulosic biomass is a desired feedstock because of the year round availability and lower costs of harvesting and transporting in comparison to crop biomass [8]. This chapter will focus mostly on upstream processing in relation to lignocellulosic biomass.

Lignocellulosic materials consist mainly of cellulose (35–50% of dry weight), Hemicellulose (25–35%) and lignin (10–25%) [78]. Cellulose is an unbranched homopolymer of glucose, usually existing in a form of highly ordered crystalline structure, which is water insoluble and hinders hydrolysis. Hemicellulose is a branched polymer and contains typically sugars such as D-galactose, D-glucose, D-mannose, D-xylose and L-arabinose. Small amounts of nonsugar components, e.g. acetyl and glucuronic groups are often present

Table 2
Characteristics of hydrolysis processes used for lignocellulosic materials [79,84].

Hydrolysis process	Advantages	Disadvantages
Dilute acid (2–5 wt%)	Low acid consumption Short processing time Acid recovery may not be required	High pressure and temperature needed Not so effective for cellulose hydrolysis Formation of undesirable by-products Corrosion of equipments
Concentrated acid (10–30 wt%)	Low temperature High yield of sugar production	High acid consumption Longer reaction time (2–6 h) compared to dilute acid hydrolysis Corrosion of equipments Acid recovery process needed, extra energy input
Enzymatic	Mild conditions (40–50 °C, pH 4.5–5.0) High yield of sugar production No formation of inhibitors	Cost of enzymes Long hydrolysis time (several days) Feedstock pretreatment required

in hemicelluloses, too. Lignin contains phenyl propane units forming a three dimensional complex structure with chemical bonds between other lignins, hemicelluloses and celluloses [79]. Lignin protects the cellulose in the cell wall and complicates enzymatic hydrolysis due to its resistance against chemical and enzymatic degradation [80].

In general, noncellulosic materials such as starch require an acidic or enzymatic hydrolysis step before fermentation. Lignocellulosic materials instead require additional pretreatment before hydrolysis and fermentation. This is because of the complex structure of lignocellulosics including cellulose crystallinity, cellulose protection by lignin, and low accessibility of enzymes to the lignocellulosic compounds. The sheathing by hemicelluloses and the degree of hemicellulose acetylation additionally hinder the biomass processing [81].

3.5.1. Pretreatment

The goal of pretreatment is to alter the structure of cellulosic biomass to improve the ability to form sugars by hydrolysis. This is achieved by breaking the lignin seal, removing lignin and hemicellulose, or increasing the porosity of the biomass. The latter is accomplished by reducing the crystallinity of cellulose or by increasing the amorphous cellulose fraction, which is the most suitable form for enzymatic action in hydrolysis [82,83]. It is crucial that the selected pretreatment method is cost efficient as it is one of the most costly steps of the conversion process from biomass to fermentable sugars. Pretreatment should improve the yield of the formation of fermentable sugars while avoiding the degradation or loss of carbohydrates and the formation of inhibitors for subsequent hydrolysis and fermentation processes.

Several physical, physico chemical, chemical and biological methods, and combinations of them have been developed for making the raw materials suitable for fermentation. Each method has its own characteristics and impacts on the whole production process. Detailed information about different pretreatment methods is found in several reviews by Jørgensen et al. [80], Hsu [81], Mosier et al. [82], Sun and Zheng [83], Taherzadeh and Karimi [84], and Kumar et al. [85].

Ammonia, dilute acid, lime, liquid hot water and steam explosion methods are potentially considered cost effective pretreatment methods [79]. In addition, during the last years the use of ionic liquids (ILs) for dissolving lignocellulosics has been examined intensively [86,87]. Selective dissolution may be achieved by selecting properly the anion and cation of the IL. The key properties of ILs to be considered are dipolarity, hydrogen bond basicity, melting point and viscosity. In addition, corrosivity, toxicity and recyclability of ILs need to be taken into consideration. Further, Xin et al. [88] have proposed supercritical carbon dioxide ($scCO_2$) as the pretreatment method and environment for wood based materials (cellulose and lignocellulosic). They studied the effect of $scCO_2$ on the supramolecular structure of cellulase and the cellulase catalyzed reactions. The cellulase activity should, however, remain when it is treated with $scCO_2$ at 10 MPa and at 50 °C for a short period (30 min). Understanding the differences between pretreatment methods and their requirements to achieve a good sugar yield for fermentation without forming a significant amount of microbial inhibitors are important factors when selecting the best available method for a certain substrate and production process. Results of upstream processing should be balanced against the impact of the fermentation and downstream processing, costs of construction, materials, chemicals and operation, and the trade off between capital, biomass and operating costs [82].

3.5.2. Hydrolysis

Cellulosic fibers and sugar polymers may be utilized for fermentation only after the hydrolysis of the pretreated material into fermentation sugars is conducted. The hydrolysis is carried out by acid or enzymatic treatments, or a combination of both. These three major hydrolysis processes used in the treatment of agricultural and wood industry wastes are illustrated in Table 2.

Acid hydrolysis is mainly performed with diluted or concentrated solutions of sulfuric or hydrochloric acid. Dilute acid pretreatment (acid concentration of 0.5–5 wt%) is operated at high pressures (around 1 MPa), at temperatures of 120–160 °C in a batch process with high solids loading (10–40 wt% dry substrate/reaction mixture) or in a continuous process at temperatures of 160–200 °C with low solids loading such as 5–10 wt% (reviewed by [83]). Dilute acid hydrolysis may be performed in two or more stages to avoid the degradation of monosaccharides and the formation of inhibitors. Countercurrent, percolation, plug flow and shrinking bed reactors are used in addition to batch reactors. Concentrated acid pretreatment is operated at low temperatures such as 40 °C, and the sugar yield is usually higher compared to the dilute acid hydrolysis [79]. Acids with a concentration of 30–70% affect cellulose efficiently but are also corrosive, toxic and hazardous [83]. Acid is added either by mixing with the biomass in a reactor, by acid percolation through the biomass bed, or by spraying acid onto the biomass. Heating of acid and biomass mixture may be done indirectly through the reactor walls or directly as a steam injection [82]. Reactors must be resistant to corrosion and for economical feasibility acids have to be recovered after the hydrolysis [89]. Neutralization to a suitable pH range is also essential before fermentation.

Enzymatic hydrolysis of lignocellulosic biomass is done by hemicellulase and cellulase enzymes (glycoside hydrolases). These enzymes are highly specific natural catalysts obtained from e.g. brown, white and soft rot fungi. Brown and soft rot fungi related enzymes are capable of depolymerizing cellulose and hemicelluloses, but lignin is only slightly modified. White rot fungi are able to degrade lignin. Enzymes involved in lignin degradation include laccases, ligninolytic peroxidases such as lignin peroxidase, manganese peroxidase and versatile peroxidase, and oxidases [90]. Cellulase enzymes are produced by bacteria, e.g. species of *Cellulomonas, Clostridium,* and *Streptomyces*, as well as by fungi such

as *Aspergillus, Humicola, Phanerochaete* and *Trichoderma* [91]. At least three kinds of cellulases are used in hydrolysis: endoglucanases, exoglucanases, also known as cellobiohydrolases, and β-glucosidases [80]. First endoglucanases degrade the low crystallinity parts of the cellulose thus providing free chain ends, which can then be modified via cellobiohydrolases by the removal of cellobiose units from the chain ends. Finally, β-glucosidases hydrolyze cellobiose to glucose. The main factors affecting the enzymatic hydrolysis are the quality and concentration of the substrate, pretreatment methods used, enzyme loading, cellulase activity and conditions of hydrolysis including pH, temperature and mixing [84]. A high concentration of cellulose and/or hemicelluloses can hinder the mass transfer and mixing, inhibit enzyme activity and thereby lower the rate of hydrolysis. The economics of the process may be improved by recovering cellulases from the solid substrate or from the liquid supernatant [92]. Other ways to improve the enzymatic hydrolysis are the immobilization of the enzymes to retain them in the reactor or combined hydrolysis and fermentation steps [84].

3.5.3. Detoxification of inhibitory compounds

During the pretreatment and hydrolysis steps some undesirable degradation products that affect negatively on enzymatic hydrolysis and fermentation steps may be formed. The sensitivity to the inhibitors varies with the microorganisms and within different strains of bacteria or yeasts. The inhibitors released during the degradation of lignocellulosic biomass include furan derivatives (furfural, 5-hydroxymethyl-furfural (5-HMF)), aliphatic acids (acetic, formic and levulinic acids), and phenolic compounds [93]. The inhibitors affecting the enzymatic hydrolysis include formic acid inhibiting cellulases and xylanases, and vanillic acid, syringic acid and syringaldehyde inhibiting xylanases [94]. In addition, glucose and cellobiose inhibit β-glucosidases and cellulases. Washing of the pretreated biomass with water before enzymatic hydrolysis increases the sugar yield [95].

Phenolic compounds, i.e. syringaldehyde, ferulic and p-coumaric acids are considered the major lignocellulosic originated inhibitors in butanol fermentation and need to be removed in the upstream processing [96]. Phenolic compounds can increase the membrane fluidity of microorganisms and thereby affect negatively the cell integrity and operation [97]. The net effects of different inhibitors may have a great impact on the fermentation, but these effects are more difficult to determine.

The concentration of inhibitors in the hydrolysates for fermentation may be decreased by the optimization of the upstream processing. Preventing their formation is, however, difficult to achieve without significant additional costs. The identification and understanding of the mode of action of inhibitory compounds present in different hydrolysates is an important research area in relation to the development of fermentation and detoxification methods for the removal of inhibitors. Many of the detoxification methods are effective only for a certain inhibitor or a class of inhibitors. Detoxification steps also increase the costs of the production process hence it is crucial to carefully evaluate the need of removing the inhibitors. Additionally, it is important to consider the costs, effectiveness and the integration of the detoxification step into the whole process.

Traditional techniques for the removal of inhibitors include biological, physical, and chemical methods and their combinations e.g. vacuum evaporation and overliming with calcium hydroxide [93]. Ion exchange resins are considered to be effective for the removal of different inhibitors from hydrolysates and are simple to operate [98]. Detoxification biological methods using peroxidase enzymes removes about 99% of the phenolic compounds [12]. Other alternatives to overcome the inhibition problem are the modification of bacterial strains to be more tolerant towards inhibitors [99], the use of immobilized cell cultures [4] or the removal of inhibitors while they are being formed by separation methods.

3.6. Downstream processing

The majority of researchers propose adsorption, liquid–liquid extraction, gas stripping and pervaporation for the recovery of butanol from the fermentation broth [13,15,100–106].

According to literature, the authors have different opinions about what technique is the most appropriate. The recovery technique should exhibit long term stability, and high selectivity and removal rate. In addition it should be simple to perform, resource efficient, harmless to the broth and low in cost. Ideally, the technique would be integrated into the fermentation step so the inhibiting product concentrations are never reached. It is of great importance that the energy required for butanol recovery is less than the energy content of the product.

Qureshi et al. [107] reported a comparative study on the energy requirement of 1-butanol recovery from the fermentation broth by adsorption, gas stripping, liquid–liquid extraction and pervaporation. They concluded that adsorption using silicalite (a lab made Al-free zeolite analogue) is the most energy efficient method (8.16 MJ kg^{-1} of 1-butanol). Oudshoorn et al. [108] reported that adsorption and pervaporation based techniques are energy efficient recovery methods. They predicted the energy requirement for the 1-butanol recovery to be <4 MJ kg^{-1} by the adsorption and pervaporation methods.

Studies on the recovery of butanol from fermentation broth by adsorption and gas stripping were conducted mainly in the 1990s. New insights are recently brought to the research on the recovery of butanol by liquid–liquid extraction. Ha et al. [109] and Simoni et al. [110] have proposed ILs as alternative solvents for conducting the extraction. The negligible vapour pressure of ILs and their ability to solubilize a wide range of organic molecules facilitate their use in the context of liquid–liquid extraction. The utilization of ILs provides several economic benefits such as no solvent loss. In addition, it may be possible to design ILs to be selective towards the extraction of certain compounds from specific matrices by the variation of the components of the IL. Ha et al. [109] observed an extraction of more than 74% of initial butanol into 1-octyl-3-methylimidazolium bis[(trifluoromethyl)sulfonyl]amide from the modelled binary aqueous solutions. Simoni et al. [110] found that 1-hexyl-3-methylimidazolium tris(pentafluoroethyl)trifluorophosphate appears to be an especially good solvent for the separation of 1-butanol from water. The use of pervaporation for the recovery of butanol continues attracting a significant amount of researchers and new information is continuously published. Consequently it is the aim of the authors to further focus on this technique.

3.6.1. Pervaporation

Kober introduced the term pervaporation for the first time in 1917. He used it to describe the selective permeation of water through a collodion bag in a publication of the Journal of American Chemical Society [111]. In the following decades pervaporation studies were conducted to further knowledge on the subject. The studies focused on the separation of liquid mixtures by pervaporation and on the development of membranes. Later, in the 1950s, pervaporation research aimed at potential industrial applications. The membranes used at that time (natural and synthetic rubbers, cellulose esters and ethers) exhibited low fluxes and separation efficiency. These negative results obstructed any large scale application of pervaporation [112]. The development of the composite GFT (Gesellschaft für Trenntechnik) membranes in the 1980s allowed for the first practical implementation of pervaporation by GFT (GFT was taken over by Carbone Lorraine that sold its pervapo-

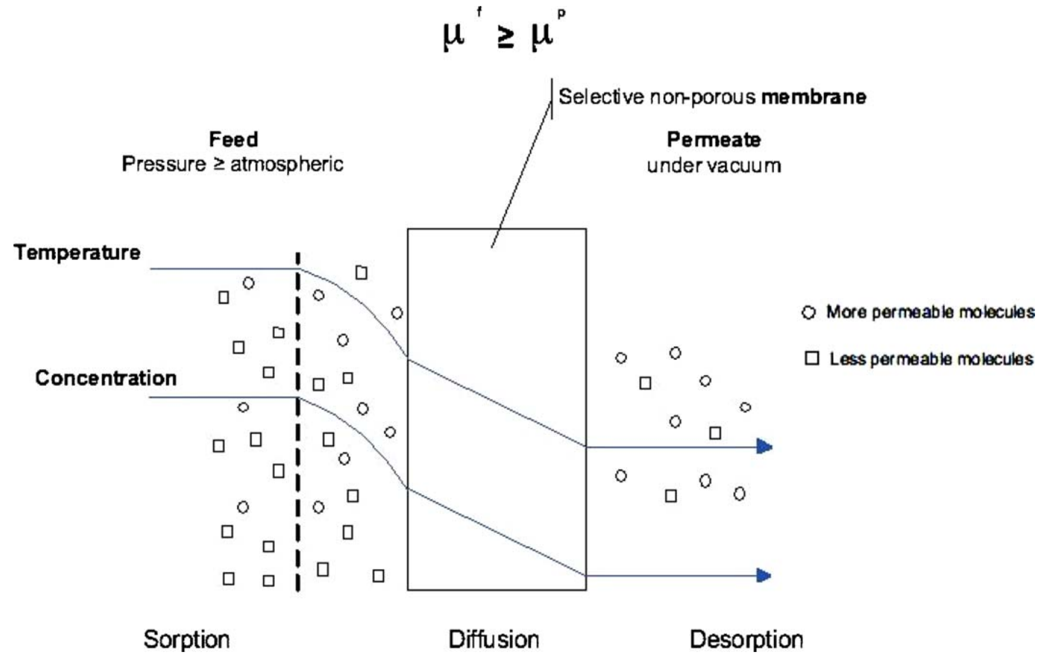


Fig. 3. Schematic diagram of the principle of pervaporation [118]; μ^f: chemical potential in the feed side; μ^p: chemical potential in the permeate side.

ration technique to Sulzer Chemtech), the dehydration of ethanol azeotropes using a cross linked poly(vinyl alcohol) (PVA) on a microporous polyacrylonitrile support [113].

3.6.1.1. Principle of pervaporation. Pervaporation is based on the molecular interactions between feed components and the membrane. In pervaporation (Fig. 3), the liquid mixture to be separated is in direct contact with one side of a dense organic membrane or molecularly porous inorganic membrane. The targeted compounds are transported through the membrane and desorbed to the permeate side as vapour. The separation occurs as a result of a gradient in chemical potential. This is achieved by differences in the partial vapour pressure of the components between the two sides of the membrane. The partial vapour pressure of the components in the feed is at saturation and is fixed by the temperature and the composition of the liquid mixture, and by the nature of the components. The other side of the membrane is kept at low pressure by applying vacuum, inert carrier gas or condensation at low temperatures.

The properties of the membrane material dictate the separation achieved in the process. If the material of the membrane is hydrophobic, the membrane will preferentially permeate organic compounds relative to water and the permeate will be enriched in the organic compounds. On the contrary, if the membrane has hydrophilic properties water will be enriched in the permeate and the organic compounds will be retained. The two main key parameters in pervaporation are membrane selectivity given as selectivity or enrichment factor, and flux. The values of these parameters depend on experimental variables such as temperature, feed compositions, membrane material, and feed side and permeate side pressures.

3.6.1.2. Pervaporation for the recovery of butanol from the ABE fermentation broth. The application of pervaporation for the recovery of butanol from the fermentation broth is based on its selective permeation through the membrane in preference to water when the nature of the selected membrane is hydrophobic. Pervaporation is especially effective when the concentration of the targeted species in the feed is low (concentration range of 200–50 000 ppm). This feature makes the application of pervaporation especially convenient for the recovery of biobutanol because of the low concentration of butanol in the fermentation broth. Pervaporation may be coupled with fermentation so that the inhibitory products from the fermentation broth are removed continuously as soon as they are formed, thereby enhancing the process productivity. Further, the pervaporation process is energy efficient and it is harmless to the fermentation broth.

A significant number of researchers have successfully conducted the recovery of n-butanol from a model or real ABE fermentation broth [15,100–106]. Table 3 shows the studies published in this field during the last ten years. The main aim of these studies was to find a stable membrane exhibiting high selectivity and high flux. Special attention was given to the fouling problems caused by the fermentation broth.

Most of the studies published use polydimethylsiloxane (PDMS) based membranes. This polymer is usually selected because of its high permeability, good selectivity and ease of preparing a variety of membrane shapes, i.e. flat or tubular membranes [103]. The performance of the PDMS membrane was further improved by incorporating silicalite into the silicone rubber [15,101,102]. Silicalites, also known as zeolites, are molecular sieves that have the capability to adsorb organic solvents such as n-butanol. Other materials used are polyether block amide (PEBA) [100,104] and poly(1-trimethylsilyl-1-propyne) (PTMSP) [103,105]. The recovery of butanol by pervaporation was also conducted using liquid membranes. It is of key importance that the solvent which impregnates the pores of the membrane is not toxic to the culture. Thongsukmak and Sirkar [106] obtained high selectivities towards butanol using trioctylamine based liquid membranes.

The fermentation broth contains impermeable components such as acids, bases, salts and sugars that may influence the performance of pervaporation. Salts and sugars can penetrate the membrane and cause membrane fouling. In addition, micro salt crystallites can physically block the pores on the permeate side [114]. The presence of electrolytes and sugars also affects both the mass transfer rate through the membrane and the pervaporation driving force. The presence of salts and sugars influences the pervaporation driving force by affecting the activity coefficients of solute and solvents, the so called salting out and sugaring out effects,

Table 3
Research published since the 2000s regarding the recovery of butanol from the fermentation broth.

Butanol concentration (wt%)	Membrane	Temperature (°C)	Notes	Reference
0.01–0.4	Silicalite-filled PDMS	25–65	Max flux of 16 g m^{-2} h^{-1} at 65 °C Max permeate concentration of 6 wt% at 25 °C	[15]
0.03–0.4	PEBA 2533	29–60	Max flux of 60 g m^{-2} h^{-1} (at max temp and conc) Separation factor: 12–23	[104]
1.5–2.5	Trioctylamine based liquid membranes	25–54	Max flux of 275 g m^{-2} h^{-1} and selectivity of 275 at 54 °C and 1.5 wt%	[106]
1–5	PEBA 2003	21–72	Max flux of 15 g m^{-2} h^{-1} and selectivity of 14 at 21 °C and 5 wt%	[100]
0.2	PTMSP	30 and 37	Membrane deterioration observed in the real ABE experiments	[105]
0.5–50	Silicalite filled silicone	30–70	Max flux of 300 g m^{-2} h^{-1} and selectivity of 90–100 at 70 °C and 10 wt%	[101]
0.5–10	Silicalite-1 silicone	78	Selectivity of 100 at 10 wt%	[102]
20	PDMS	26–62	Max flux and selectivity of 250 g m^{-2} h^{-1} and 55 at 62 °C	[103]
	PTMSP		Max flux and selectivity of 2500 g m^{-2} h^{-1} at 62 °C and 135 at 37 °C	

ABE: acetone–butanol–ethanol; PDMS: poly(dimethylsiloxane); PEBA: polyether-*block*-polyamides; PTMSP: poly(1-trimethylsilyl-1-propyne).

respectively. Salting out is the phenomenon by which the solubility of one solute in a solvent is altered by the presence of another solute. The compound is said to be salted out by the electrolyte if its solubility is reduced in the presence of a salt. Analogously, an organic solvent that is miscible with water in all proportions can form a two phase system with water when sugars are added to the solution [115]. Further, salts and sugars increase the density and viscosity of the feed solution. As a consequence, the transport rate should be slower. The effect of salts and sugars on the pervaporation properties is a balance between the salting out and sugaring out effects, and the retarding effect of the increased viscosity and density. Few authors have studied the influence of electrolytes on the pervaporation of butanol. García et al. [116,117] did not observe a significant influence of the electrolyte (NaCl) on the pervaporation performance of PDMS based membranes (CMX-GF-010-D and PERTHESE® 500-1) towards butanol.

Pervaporation using hydrophobic membranes might not be enough for concentrating butanol for direct reuse [118]. Further purification is also possible by conducting pervaporation using hydrophilic membranes [119–125]. Table 4 shows a selective list of publications and the type of membranes used in the dehydration of *n*-butanol by pervaporation using polymeric and mixed matrix membranes. The dehydration of *n*-butanol by pervaporation is conducted at different feed concentrations and temperatures using commercially and specific laboratory produced membranes mainly consistent of crosslinked PVA. The polar group in the PVA material is very effective in dehydration since the alcohol group has a high polarity and interacts strongly with water through hydrogen bonding. Further, it shows chemical stability, membrane forming ability and heat resistant properties [119]. Garcia et al. [120] used a composite PVA membrane supplied by GKSS Forschungszentrum (Geesthacht, Germany). The top active layer of the PVA membrane contains titanium dioxide as nanoparticles to add stability to the membrane. The concentration of water in the permeate was 97.6 wt% at the optimized experimental conditions. PERVAP 2201 has higher degrees of crosslinking and chain packing than PERVAP 2510, while PERVAP 2510 has stronger hydrophilicity than PERVAP 2201 [126]. Furthermore, PERVAP 2201 exhibits high selectivity but low fluxes [127]. Other alternative polymers suggested are chitosan (CS) [121] polyamide polyimide and polyimide membranes [122,123].

Some of the challenges that research faces in order to make the implementation of pervaporation to the ABE fermentation viable are the decrease of energy input, the reduction of capital costs and the development of membranes that exhibit higher selectivities and fluxes and have stability towards the conditions of the real fermentation broth. A better understanding of multicomponent systems and of the transfer of mass and heat will have a positive impact on achieving those aims. A decrease of energy input may be achieved by studying possibilities for energy recovery or alternative condensation techniques. Research on materials plays an important role as it opens the door for creating new membranes from cheap materials that are effective and stable. If the membranes exhibit high fluxes the membrane area is reduced and the capital costs are decreased.

4. Integration of the process: biorefinery thinking

The American National Renewable Energy Laboratory [128] defines biorefinery as "A facility that integrates biomass conversion processes and equipment to produce fuels, power, and chemicals from biomass. The biorefinery concept is analogous to today's petroleum refineries, which produce multiple fuels and products from petroleum." An example of the biorefinery concept is illustrated in Fig. 4. Kamm and Kamm [129] classified biorefineries as lignocellulosic feedstock biorefinery, whole crop biorefinery and green biorefinery. Other classifications are proposed by Naik et al. [1] and Demirbas [130].

The goal of a biorefinery is to convert raw materials efficiently into products, i.e. chemicals, fuels, materials and energy, by using economically viable processing methods. In doing so the use of resources needs to be optimized. Those resources are not only raw materials but also consumables, water, energy, working space, labour, and by products. Further, the ultimate aim of a biorefinery is to offer products to replace the ones coming from oil refineries. The concept of biorefinery brings new possibilities for innovations and market opportunities. The benefits of biorefineries are numerous because of the diversity in feedstocks and products.

The economics of the processes used in biorefineries may be improved by reducing the investment costs. Low investment costs are achieved using small reactors with mechanical simplicity and high productivity, while low operating costs require continuous processes, use of low cost substrates, near or complete substrate utilization, low energy input and use or elimination of byproducts [131]. Reducing the environmental impact of the process is also of a great importance. Abbasi and Abbasi [132] have investigated biomass energy and the environmental impacts associated with its production and utilization. The consumption of nonrenewable energy resources during biorefinery processing should be minimized and the complete and efficient biomass use maximized

Table 4
Selected studies regarding the dehydration of butanol by pervaporation.

Concentration (wt%)	Membrane	Temperature (°C)	Notes	Reference
$5 < C_w < 20$; $5 < C_{DCM} < 45$; $50 < C_b < 90$	PVA – TiO_2/PAN/PPS	30–50	Water flux: 29–313 g m^{-2} h^{-1}; enrichment factor: 6–29	[120]
$C_w = 11.6$; $C_b = 82.6$; $C_{2,3\ butanediol} = 5.8$	CS/PVDF composite	50	Final feed water concentration 0.6 wt% after 15 h	[121]
$C_w = 15$; $C_b = 85$	PAI/PEI hollow fiber membranes	60	Water fluxes: 523–758 g m^{-2} h^{-1} Selectivity: 460–1296[a]	[122]
$C_w = 5$; $C_b = 95$	Ceramic-supported polyimide	95	Water fluxes: 1000–6000 g m^{-2} h^{-1} Selectivity: 360	[123]
$C_w = 10$; $C_b = 90$	PVA/SA blend hollow fiber composite	45	Total flux: 475–625 g m^2 h^{-1} Selectivity: 700–800[a]	[119]
$C_w < 20$; $C_b < 80$	PERVAP 2510 (PVA-PAN-PPS)	60–100	Max selectivity of 7 at 100 °C, $C_w = 2$ wt% Max butanol flux of 900 g m^2 h^{-1} at 100 °C, $C_w = 16$ wt%	[124]
$85 < C_b < 95$; $5 < C_w < 15$	PVA crosslinked with glutaraldehyde	30–50	Max butanol flux of 900 g m^{-2} h^{-1} at $C_b = 85$ wt% at 50 °C	[125]

b: butanol; CS: chitosan; DCM: dichlromethane; PAI: polyamide–imide polyetherimide; PAN: polyacrylonitrile; PEI: polyetherimide; PPS: polyphenylene sulfide; PVA: poly(vinyl alcohol); PVDF: polyvinyledenefluoride; SA: sodium alginate; TiO_2: titanium dioxide; w: water.
[a] Depending on membrane morphology or composition.

[78]. To gain energy efficiency and minimized water usage, processes should be operated with large biomass particle size, high solid concentrations and with integrated surplus heat/steam usage for pretreatment from some other processes [80]. Different sources of energy and options for energy recovery need also to be considered. One optional energy source is the anaerobic conversion of waste waters to biogas, which is burned to generate steam and electricity or sold as a byproduct. The energy released from some processes within the biorefinery should be evaluated as a potential energy input in other processes in the system.

The utilization of raw materials and by products gives other opportunities to improve the ecoefficiency of the process. It is important to mention that bio industries can combine their material flows in order to reach a complete utilization of all biomass components. The residue from one bio industry becomes an input for other industries, giving rise to integrated bio industrial systems.

The ecoefficiency of the biobutanol production can benefit from the biorefinery concept. Biobutanol would be the main product. However, utilization and market opportunities for the byproducts and compounds from the waste streams should be evaluated. Table 5 shows the possible products and applications of a biorefinery using lignocellulosic feedstock and producing butanol as the main product [133,134].

The use of highly productive reactors in the biobutanol process allows the reuse of the cell mass. The biomass generated from the process may be used as an animal feed. Another example is given by the lignin dissolved into black liquor that after pulping may be gasified to produce syngas. The resulting syngas can be further converted to fuels and chemicals as well as electricity and process steam [98]. Enhancement of resources utilization is done by implementing recovering technologies in the system. For example, the inhibitors formed in fermentation processes may be separated from hydrolysates before the fermentation unit thereby enhancing the fermentation and product yields. Inhibitors may as well be utilized as valuable chemicals. The gases formed during the fermentation may also be reclaimed. CO_2 gas may be recycled and used as a substrate or sold for other applications while H_2 may be separated from the gas mixture of CO_2 and H_2 and used as a fuel. CO_2 and H_2 can also be converted to methanol and further to e.g. dimethylcarbonate, dimethyl ether and carbonic esters [135,136]. The utilization of side and waste streams must be economically viable.

In order to reduce the environmental impact of biorefineries, waste streams need to be considered. The amount and toxicity of those waste streams may be reduced significantly thanks to the development in bioreactor design and integration of the in situ recovery with the fermentation [77]. Waste streams may also con-

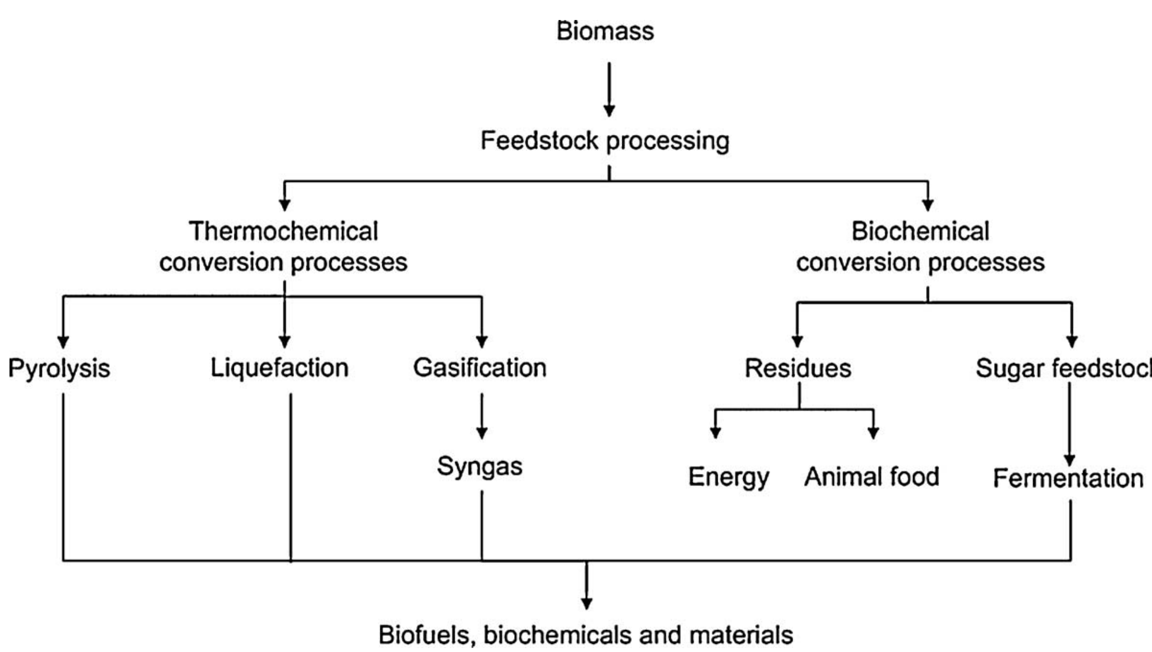


Fig. 4. Schematic diagram of the biorefinery concept.

Table 5
Possible products and applications of the butanol biorefinery based on lignocellulosic raw materials.

Biomass fraction	Compound	Products and usage
Cellulose	Hexoses, e.g. glucose	Acetone, butanol, and ethanol, H_2, CO_2: fuels and chemicals
	HMF	Compound used in food industry FDCA: starting compound for production of polyamides and polyesters (Replacement of terephtahlic acid)
	Levulinic acid	Softener, solvents, lubricants, chemicals and polymers
Hemicellulose	Pentoses, e.g. xylose	Acetone, butanol, and ethanol, H_2, CO_2: fuels and chemicals
	Furfural (2-furaldehyde)	Selective solvent, flavor ingredient
Lignin	Kraft lignin	Heat production, activated carbon, carbon black substitutes, phenolic resin components, reinforcer in rubber, raw materials for methylsulfonate production
	Lignosulfonates (from sulfite pulping process) crude spent liquor lignosulfonates	Agricultural dispersants and emulsifiers, dust suppression and road stabilization, granulation and agglomeration, grinding aids, extenders for feed molasses, feed and pellet binders
	Refined lignosulfonates	Cement manufacture, concrete admixtures, tanning agent, gypsum board manufacture, refractory ceramics and clays, phenolic resins, dispersants for dye and pigment, oil well drilling fluids, protein precipitants, lead acid storage pattery plates
Extractives	Conjugated linoleic acids	Antioxidants, antitumor properties, dietary supplements
	Flavonoids	Medical applications
	HMR	Dietary supplement
	Phenolic compounds (from e.g. knotwood)	Technical or biological antioxidants, functional foods, pharmaceuticals, natural biocides e.g. bactericides, fungicides, pesticides
	Tannins	Medical applications
	Sterols	Sitosterol (lowering of cholesterol level of blood)
	Vanillin	Flavour in food industry, aroma in cosmetics, chemical intermediate
	Wood and bark tars and resins	Turpentine, tall oil products, tannins, stilbenes, wood bark tars and resins.

FCDA: furan-2,5-dicarboxylic acid; HFM: 5-hydroxymethyl-2-furaldehyde; HMR: hydroxymatairesinol.

tain nutrient components such as proteins, organic acids, vitamins (particularly Vitamin B2 and B12), as well as saccharides, which are an excellent animal feed [33].

Finally it is important to note that the potential of butanol biorefinery based on lignocellulosic biomass will not be fully realized until technologies that would allow the complete utilization of lignocellulosic materials are developed. The creation of those technologies should be based on the 12 principles of green chemistry and engineering. Consequently research plays an important role in the development of biorefineries and in the improvement of their sustainability.

5. Conclusion

The growing interest for biobutanol as fuel opens new opportunities for innovation and market possibilities through the biorefinery concept. The industrial production of butanol by the ABE fermentation is not currently viable due to the low yield encountered, sluggish fermentations, uneconomical product recovery and problems with the degeneration of production organism, and phage infections. This paper stresses the idea of improving the efficiency of the fermentation by altering the pretreatment of the raw material, fermentation itself, product recovery and purification processes.

The review concludes the importance of research in producing biobutanol by the ABE fermentation feasible, and in the development of biorefineries and in the improvement of their sustainability. This review points out that the present research is mainly focused on the field of understanding the physiological principle of solvents production and metabolic engineering and butanol recovery techniques. Studies on other fields such as butanol tolerance are lacking. This paper indicates that further efforts on microbiology and genetic engineering, materials science and process and bioprocess engineering may alleviate or solve the problems found in the biobutanol process. Future research should focus on sustainable assessment, efficient use of substrates including waste and by products, unlocking of problems caused by inhibition, development and improvement of process technologies involving all process steps, utilization of valuable compounds from effluents and on the development of new products. Process engineering should follow green chemistry and engineering principles in order to reach sustainability. Special attention should be given to the resource efficiency of the biobutanol production. Finally the knowledge obtained for the biobutanol production may be used for the production of other high chain alcohols such as pentanol.

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